Synthesis and Hypolipidemic and Antiplatelet Activities of α-Asarone Isomers in Humans (in Vitro), Mice (in Vivo), and Rats (in Vivo)

Janusz Popławski,[§] Bożena Łozowicka,[§] Alina T. Dubis,[§] Barbara Lachowska,[§] Stanisław Witkowski,[§] Danuta Siluk,[#] Jacek Petrusewicz,[#] Roman Kaliszan,[#] Jacek Cybulski,[‡] Małgorzata Strzałkowska,[‡] and Zdzisław Chilmonczyk*,[†]

Institute of Chemistry, University of Białystok, J. Piłsudskiego 11/4, 15-443 Białystok, Poland, Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland, Pharmaceutical Research Institute, Rydygiera 8, 01-793 Warszawa, Poland, and Drug Institute, Chełmska 30/34, 00-725 Warszawa, Poland

A series of α-asarone isomers was synthesized and investigated for their hypolipidemic and antiplatelet activity. Considering the hypolipidemic activity in rats at a dose of 80 mg/kg/day, some isomers were more potent than clofibrate at 150 mg/kg. Compound **3** was one of the most active agents elevating the HDL cholesterol level by 56% and lowering the LDL cholesterol level by 46.8% in rats after 7 days of administration. The activities of the platelet aggregation test in vitro were significant but lower than those of the reference substances (indomethacine and acetylsalicylic acid). In the pulmonary thromboembolic in vivo test in mice, two compounds (α-asarone (**6**) and compound **4**) produced significant antithrombotic effects at 100 mg/kg, namely 44% and 52% protection against lung microembola, respectively. α-Asarone derivatives form a new group of potential hypolipidemic and/or antithrombotic agents. The compounds **3**, **4**, and **6** may serve as lead substances whose structural modifications may result in original drugs.

Introduction

Cholesterol is required in the assembly of membranes and performs other important biological functions. However, when plasma cholesterol exceeds the level required for these functions, development of atherosclerosis and related diseases takes place. Hyperlipoproteinemia, and especially hypercholesterolemia, are two risk factors connected with the development of atherosclerosis and thrombosis. Particularly, a high LDL cholesterol level plays an important role in atherogenesis process.[1] The dietary and pharmacological lowering of elevated plasma LDL cholesterol appears to be one of the methods to reduce the development of atherosclerosis.[2]

Thrombocytes and their functions are also under particular investigation from the viewpoint of the pathogenesis of atherosclerosis, thrombosis, and acute coronary syndromes.[3] It was demonstrated that in animals with thrombocytopenia, atherosclerotic lesions are smaller or plaques are not observed.[4] It was also shown that in individuals with atherosclerotic alterations, the platelets exhibit a higher aggregability in vivo.[5] Excess lipoprotein concentration enhances platelet sensitivity to the activating agents. It is well-established that antiplatelet treatment lowers vascular disease mortality and the risk of myocardial infarction and stroke incidence in patients with unstable ischemic heart disease.

Since it has been discovered that acetylsalicylic acid inhibits the activity of thrombocytes, new antiplatelet agents have been of particular interest. Substances affecting the platelet function belong to different chemical groups and exhibit diverse mechanisms of action. Two main groups can be distinguished among antiplatelet drugs: agents affecting biotransformation of arachidonic acid and drugs with other mechanisms of action.[6]

Chemical structures of the agents modifying platelet functions are comprised of phenol, phenylethanol, imidazole, triazole, thiazole, pyrazolpyridine, indole, carbamoilpiperidine, piperazine, and pyrazine. Among substances of plant origin, the flavonoids that are benzo-γ-pyrone derivatives are of importance because of their inhibitory effect on capillary permeability and antiinflammatory, spasmolytical, diuretic, and antiaggregatory activity.[7] Natural procyanidines have also been shown to exert antiplatelet activity.[8]

α-Asarone (**6**), occurring in nature in the common asarabacca root *Asarum europaeum* L. and calamus *Acorus calamus* L. roots, exhibits a wide spectrum of biological activity.[9] It was isolated from the *Guatteria guameri* plant growing in Southeast Mexico. The plant has been applied in traditional Mexican human medicine in the treatment of symptoms of hypercholesterolemia and cholelithiasis.[10]

Gomez et al.[11] studied the effect of α-asarone in rats with hypercholesterolemia. They found that α-asarone administered at the dose of 4−25 mg/kg/day lowered triglyceride and cholesterol levels in plasma for 40−60%. Other investigators reported various other biological effects of α-asarone: sedating, neuroleptic, spasmolytic,
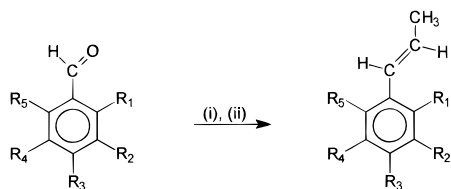
* To whom correspondence should be addressed. Tel: +48228515229. Fax: +48228410652. E-mail: chilmon@il.waw.pl.
§ University of Białystok.
# Medical University of Gdańsk.
‡ Pharmaceutical Research Institute.
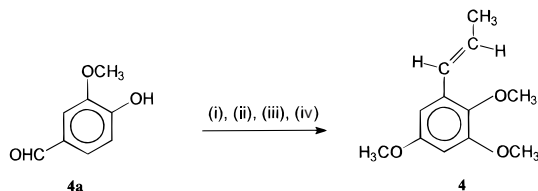† Drug Institute.

Scheme 1. Synthesis of Asarone Derivatives **1−3** and **6**[a]

(**1a**), R_1, R_2, R_3 = -OCH$_3$
(**2a**), R_1, R_3, R_5 = -OCH$_3$
(**3a**), R_2, R_3, R_4 = -OCH$_3$

(**1**), R_1, R_2, R_3 = -OCH$_3$
(**2**), R_1, R_3, R_5 = -OCH$_3$
(**3**), R_2, R_3, R_4 = -OCH$_3$
(**6**), R_1, R_3, R_4 = -OCH$_3$

[a] Reagents and conditions: (i) CH$_3$CH$_2$Br, Mg, THF(anhyd), 3 h; (ii) CuSO$_4$(anhyd), toluene, 2 h, 110 °C.

Scheme 2. Synthesis of Compound **4**[a]

[a] Reagents and conditions: (i) H$_2$C=CHCH$_2$Br, K$_2$CO$_3$, acetone, 2 h, 50 °C; (ii) oil bath, 8 h, 180 °C; (iii) H$_2$O$_2$, KOH, H$_2$O, 24 h; (CH$_3$)$_2$SO$_4$, 10% water solution KOH, 1 h, 80 °C; (iv) T, KOH, ethanol(anhyd), 24 h, 70 °C.

antiulcerogenic, and antiatherogenic.[12,9b] Also antihelminthic, teratogenic, and carcinogenic actions were noted.[13]

It appeared interesting to test a series of α-asarone isomers with regard to their influence on HDL and LDL cholesterol levels, as well as their in vitro antiaggregatory and in vivo antithrombotic activity.

Chemistry

The compounds employed in this study were synthesized according to Schemes 1−3. Scheme 1 illustrates the synthesis of 4-(prop-1-enyl)-1,2,3-trimethoxybenzene (**1**), 3-(prop-1-enyl)-1,3,5-trimethoxybenzene (**2**), and 5-(prop-1-enyl)-1,2,3-trimethoxybenzene (**3**) by using typical conditions of the Grignard reaction. Aldehydes **1a**, **2a**, or **3a** were treated with ethylmagnesium bromide in dry THF to afford appropriate trimethoxy(1-hydroxypropyl)benzene. Resulting alcohols were then subjected to dehydration in the next step. The yield of reaction strongly depends on the temperature and duration of the elimination process.

The preparation of compound **4** was achieved by formation of allyl ether from vaniline (**4a**), Claisen rearrangement followed by the Dakin reaction, and methylation of the phenol formed. Isomerization of the double bond catalyzed by base (KOH) was the final step (Scheme 2). The best result was achieved using ethanol as a solvent in the isomerization process.

The isomer **5** was synthesized by lithiation of 1,2,4-trimethoxybenzene (**5a**) by means of *n*-buthyllithium[14] in the presence of *N,N,N,N*-tetramethylethylenediamine (TMEDA), followed by treatment with propionaldehyde. The resulting alcohol was subjected to dehydration by anhydrous CuSO$_4$ (Scheme 3).

In all cases the elimination reaction gave a 93:7 mixture of the *E* and *Z* isomers of olefins **1−5**. Column

Scheme 3. Synthesis of Compound **5**[a]
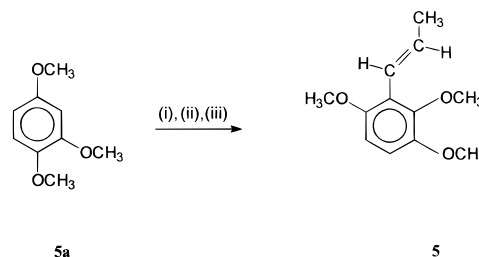
[a] Reagents and conditions: (i) *n*-BuLi, TMEDA; (ii) CH$_3$CH$_2$CHO; (iii) CuSO$_4$, toluene, 2 h, 110 °C.

chromatography or recrystallization from petroleum ether separated the two isomers completely.

Biological Results

Hypolipidemic Activity. The hypolipidemic activity study in rats at 80 mg/kg/day showed that some isomers of α-asarone were more active than the reference clofibrate at its therapeutic dose (150 mg/kg/day; the results were in agreement with the results obtained by Diaz et al.[10b]). The cholesterol and trigliceride levels were decreased by 24.1% and LDL cholesterol increased by 6.7% (Table 1).

α-Asarone at a dose of 80 mg/kg caused 2.3% increase of the total cholesterol level. At the same time HDL cholesterol was increased by 56.7% and LDL cholesterol was decreased by 43.2%. Compounds **1−4** showed similar hypolipidemic effects affording 15% reduction of serum cholesterol. Compound **2** exhibited the maximum hypotriglycemic activity. The data from the rat serum lipoprotein fractions study (Table 1) for compounds **1−4** show that HDL cholesterol levels were elevated and LDL cholesterol levels were reduced. The compounds increased the HDL cholesterol level significantly higher than clofibrate. The isomer **3** exhibited the best hypolipidemic activity as measured by 56% elevation and 56% reduction of HDL and LDL cholesterol levels, respectively.

Effect on Platelet Aggregation. α-Asarone and two of its derivatives were tested in the study. For the sake of comparison, selected nonsteroidal antiinflammatory drugs were also tested. Indomethacine and acetylsalicylic acid served as such reference drugs.

Indomethacine was used at concentrations of 5, 10, 15, 20, and 25 μM. At the concentration of 25 μM it inhibited ADP-induced aggregation by 55%. The mean IC$_{50}$ value for indomethacine was 2.4×10^{-5} M. Acetylsalicylic acid was used at concentrations of 100, 150, 200, and 250 μM. At the highest studied concentration it inhibited platelet aggregation by 56% (Table 2). Its IC$_{50}$ value was 2.12×10^{-4} M.[15]

Asarones were tested at five concentrations: 100, 150, 200, 250, and 300 μM. Similar to the indomethacine test, the aggregation-inducing substance was ADP. α-Asarone at 300 μM concentration caused 47.0 ± 19.0% inhibition of aggregation. Compounds **2** and **4** added at analogous concentrations gave 17.5 ± 4.0% and 38.3 ± 13.8% inhibition, respectively (Table 2).

Effect on Pulmonary Thromboembolism in Mice. At first the reference substances acetylsalicylic acid (ASA) at a dose of 25 mg/kg and ketorolac at a dose of 100 mg/kg mouse body weight were tested after ip

Table 1. Alteration of Blood Lipoprotein Levels by α-Asarone Isomers

compound	dose (mg/kg)	total cholesterol (mg/dL)	LDL cholesterol (mg/dL)	HDL cholesterol (mg/dL)	triglycerides	LDL/HDL	atherogenic index
normal diet[a]		$66.8 \pm 2.00^{**}$	$8.9 \pm 1.11^{**}$	$45.2 \pm 1.43^{**}$	$76.1 \pm 3.90^{**}$	0.20	0.52
cholesterol diet (CD)		110.9 ± 5.70	43.2 ± 2.91	29.0 ± 1.43	125.1 ± 6.80	1.49	2.82
clofibrate + CD	150	$85.8 \pm 5.50^{**}$	46.1 ± 5.44	$22.1 \pm 2.26^{**}$	$91.6 \pm 12.20^{**}$	2.08	2.87
α-asarone + CD	80	113.6 ± 9.20	$24.4 \pm 6.70^{**}$	$45.5 \pm 2.37^{**}$	$219.3 \pm 26.7^{**}$	0.54	1.45
1 + CD	80	96.6 ± 6.80	30.1 ± 8.69	$42.8 \pm 3.20^{**}$	124.6 ± 10.5	0.70	1.26
2 + CD	80	98.1 ± 6.42	36.9 ± 6.90	$37.9 \pm 2.75^{**}$	116.2 ± 5.84	0.97	1.58
3 + CD	80	93.4 ± 11.0	19.8 ± 5.60	$45.3 \pm 2.43^{**}$	135.7 ± 14.01	0.44	1.06
4 + CD	80	97.9 ± 3.70	35.8 ± 4.22	$39.2 \pm 2.43^{**}$	140.2 ± 10.31	0.91	1.50
5 + CD	80	115.1 ± 9.30	48.2 ± 4.30	30.2 ± 2.81	$161.5 \pm 18.40^{*}$	1.59	2.80

[a] Group receiving laboratory chow and the vehicle oil. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs cholesterol diet group, $n = 9-10$.

Table 2. Percent Inhibition of Human Platelet Aggregation Induced by ADP after Incubation with Acetylsalicylic Acid, α-Asarone, and Its Isomers[a] in Vitro

	% inhibition (mean $\pm$ SEM)[c]			
concn of agent (μM)	acetylsalicylic acid[b]	compd **2**	compd **4**	compd **6**
100	31.2 ± 5.1	15.9 ± 4.3	13.0 ± 5.4	4.0 ± 2
150	37.1 ± 4.9	19.0 ± 6.1	25.4 ± 10.8	14.7 ± 10
200	48.5 ± 6.7	31.0 ± 6.4	35.4 ± 10.3	40.3 ± 17
250	56.4 ± 5.3	7.5 ± 3.6	38.3 ± 15.2	30.3 ± 17
300		17.5 ± 4.0	38.3 ± 13.8	47.0 ± 19

[a] The percentage inhibition of platelet aggregation = $[(C - A)/C] \times 100\%$, where C is the percentage aggregation of PRP induced by 10 μM ADP after incubation with the vehicle (control) and A is the percentage aggregation of PRP induced by 10 μM ADP after incubation with the studied compounds. [b] Data from ref 15. [c] The data are means from 4–5 experiments.

Table 3. Protective Effect of Acetylsalicylic Acid, Ketorolac, α-Asarone (**6**), and Its Isomers on Mice after Thrombotic Challenge Induced by a Mixture of Collagen and Epinephrine

agent	dose (mg/kg)	no. killed or paralyzed/ no. tested	% protected
control		4/24	15
sodium acetate			
acetylsalicylic acid	25	28/51	45[a]
control		27/35	23
DMSO buffer			
ketorolac	100	16/24	33
control		45/55	18
Miglyol-812			
α-asarone (**6**)	50	13/13	0
100	10/18	44[a]	
1	100	14/14	0
2	100	30/40	25
3	100	17/21	19
4	100	12/25	52[b]

[a] Significantly different from controls, $p < 0.05$; data from ref 15. [b] Significantly different from controls, $p < 0.01$.

administration. ASA prevented pulmonary microembolism in mice at 45%, while ketorolac gave 33% protection (Table 3).

α-Asarone administered intraperitoneally at a dose of 100 mg/kg gave 44% protection ($p < 0.05$), while a dose of 50 mg/kg did not give any statistically significant protection (Table 3). The strongest antithrombotic activity among the asarone isomers was found for compound **4** (100 mg/kg) which showed 52% ($p < 0.01$) protection (Table 3).

Discussion

Our data demonstrated that a series of α-asarone isomers are potent hypolipidemic agents in rats (Table 1). The present study shows that some α-asarone isomers decrease and some increase the level of total cholesterol and LDL cholesterol as compared to the controls. One of the major features of these agents is that they lower LDL and elevate HDL contents. HDL cholesterol was significantly increased in the cases of compounds **1**–**4** which appear to be more active than clofibrate at its therapeutic dose of 150 mg/kg/day.[16]

The HDL fraction leads cholesterol out of the tissue and protects against atherosclerosis.[17] The LDL fraction is generally thought to carry cholesterol to the tissues and is responsible for the atherogenesis process. Decreasing the ratio of the plasma level of LDL to that of HDL seems to play an important role in reducing the risk of atherosclerosis. Compound **3** showed a 5 times lower value of this ratio than clofibrate (Table 1). Similarly, compounds **1**, **2**, and **4** had that value in the range of 0.7–1, whereas the LDL/HDL ratio for clofibrate is 2.08.

The analysis of the atherogenic index (total cholesterol − LDL/HDL)[18] (Table 1) showed that compound **3** was endowed with potent hypolipidemic activity. Its derivatives will be the subject of a further study.

Three asarone isomers were investigated (α-asarone (**6**) and compounds **2** and **4**) in the in vitro platelet aggregation test. The compounds did not exert evident antiaggregatory activity in vitro against the ADP-induced platelet aggregation. The observed effects are much weaker as compared to those of indomethacine. Nevertheless, the absence or a low activity in vitro does not disqualify the substances tested as potential antiplatelet agents. Ticlopidine, one of the most frequently used antiplatelet drugs, also does not show any in vitro activity.[19]

On the basis of the pharmacological tests carried out, we conclude that α-asarone (**6**) and isomer **4** exert antithrombotic action in vivo, although the activity of the agents is significantly lower than that of acetylsalicylic acid. Compounds **1**–**3** did not show any antithrombotic activity in our in vivo studies.

α-Asarone derivatives appear to be a new promising original group of potential hypolipidemic and antithrombotic drugs and deserve further investigation. The compounds **3**, **4**, and **6** may serve as lead substances whose structural modifications may result in potent original antithrombotic drugs.

Experimental Section

Chemicals and Reagents. A series of newly synthesized α-asarone isomers (**1**–**5**) was subjected to pharmacological studies. Other substances used in the study were purchased from the following sources: α-asarone from Aldrich-Chemie (Steiheim, Germany), acetylsalicylic acid (ASA) and ketorolac from Polpharma S.A. (Starogard Gdański, Poland), indometha-

cine from Polfa Kraków (Kraków, Poland), clofibrate from Polfa Grodzisk (Grodzisk Mazowiecki, Poland), miglyol 812 from Caelo (Hilden, Germany), and ADP, collagen and epinephrine from Sigma (St. Louis, MO). Sodium chloride and sodium acetate were obtained from Polish Chemical Reagents (Gliwice, Poland), dimethyl sulfoxide from Ubichem Ltd. (Hampshire, U.K.), sodium cholate from Aldrich (Poznań, Poland) and murigram from Lomianki (Poland). All used kits were purchased from BioMerieux (Poland).

Chemical Methods. Melting points were determined on a Köffler apparatus of the Böetius type and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200F spectrometer using CDCl$_3$ solution with TMS as internal standard (chemical shifts in δ, ppm). IR spectra were recorded on a Nicolet Magna 550 FTIR spectrometer in chloroform solutions. The UV spectra were collected on a Hewlett-Packard UV–vis diode array spectrophotometer 8452A. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on silica gel 70–230 mesh ASTM (Merck). Thin-layer chromatograms were developed on aluminum TLC sheets precoated with silica gel F$_{254}$. The spots were visualized with 50% sulfuric acid after heating. All the solvents were dried and freshly distilled prior to use.

Synthesis of 4-((E)Prop-1-enyl)-1,2,3-trimethoxyben-zene (1), 2-((E)Prop-1-enyl)-1,3,5-trimethoxybenzene (2), and 5-((E)Prop-1-enyl)-1,2,3-trimethoxybenzene (3). 9.1 mL (0.1 mol) of 1-bromoethane in 20 mL of THF was added dropwise to a mixture of 2.4 g (0.1 mol) of magnesium turnings in 15 mL of dry THF. The solution of 0.02 mol of trimethoxy-benzaldehyde (**1a**, **2a** or **3a**) in 20 mL of THF was added dropwise after the formation of Grignard reagent. After 3 h of stirring, 50 mL of methanol was added and the resulting solution was diluted with water and extracted with ether. The organic extract was washed with water and dried over MgSO$_4$. The mixture was filtered and concentrated under a reduced pressure. The crude product was used without any purification in the next step.

A solution of crude alcohol (0.02 mol) in 50 mL of dry toluene was treated with 4.8 g (0.03 mol) of CuSO$_4$.[20] The reaction mixture was refluxed 2 h. An inorganic material was filtered off and washed with toluene. After the evaporation of the solvent, the residue was purified by silica gel column chromatography (elution with hexane/ether, 3:7, v/v) giving **1**, **2** or **3**. Compound **2** was recrystallized from petroleum ether (bp 60–80 °C).

Compound **1**: mp 25–26 °C; IR ν(CHCl$_3$) 3020, 1600, 1100 cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ 7.10 (d, 1H, $J = 8.70$ Hz); 6.63 (d, 1H, $J = 8.70$ Hz); 6.58 (dq, 1H, $J_1 = 1.66$ Hz; $J_2 = 15.9$ Hz); 6.13 (1H, dq, $J_1 = 6.57$ Hz, $J_2 = 15.9$ Hz); 3.88 (s, 3H); 3.86 (s, 3H); 3.85 (s, 3H); 1.89 (3H,dd, $J_1 = 1.67$, $J_2 = 6.57$); ^{13}C NMR 152.4 (C); 150.8 (C); 142.2 (C); 125.0 (C); 124.9 (CH); 124.8 (CH); 120.3 (CH); 107.6 (CH); 60.8 (CH$_3$); 60.6 (CH$_3$); 55.8 (CH$_3$); 18.6 (CH$_3$); UV λ_{max} (nm) 264 ($\epsilon = 15300$); MS $m/z = 208$ (M$^+$, 100%), 193 (18%), 179 (24%), 133 (31%). Compound **2:** mp 72–73 °C; IR (CHCl$_3$) 3022, 1605 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 6.59 (d, 1H, $J = 16.1$ Hz); 6.49 (dq, 1H, $J_1 = 5.45$ Hz, $J_2 = 16.1$ Hz); 6.15 (s, 2H); 3.83 (s, 6H) 3.82 (s, 3H); 1.91 (d, 3H, $J = 4,9$ Hz); ^{13}C NMR 159.3 (C); 158.7 (C); 127.8 (CH); 120.9 (CH); 108.8 (C); 90.6 (CH); 55.6(CH$_3$); 55.2 (CH$_3$); 20.0 (CH$_3$); UV λ_{max} (nm) 268 ($\epsilon = 28953$), 222 ($\epsilon = 38248$); MS $m/z = 208$ (M$^+$, 100%), 193 (13%), 179 (61%), 151 (25%). Compound **3**: mp. 17–18 °C; IR (CHCl$_3$) 3022, 1610 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 6.56 (s, 2H); 6.34 (dq, 1H, $J_1 = 1.28$ Hz, $J_2 = 15.7$ Hz); 6.17 (dq, 1H, $J_1 = 6.2$ Hz, $J_2 = 15.7$ Hz); 3.87 (s, 6H); 3.84 (s, 3H); 1.89 (dd, 3H, $J_1 = 1.23$ Hz, $J_2 = 6.22$ Hz); ^{13}C NMR 153.2 (C); 133.7 (C); 130.8 (CH); 125.2 (CH); 129.8 (C); 102.7 (CH); 60.8 (CH$_3$); 55.9 (CH$_3$); 18.3 (CH$_3$); UV λ_{max} (nm) 266 (25932); MS $m/z = 208$ (M$^+$, 100%), 193 (15%), 179 (57%), 151 (20%), 121 (16%).

Synthesis of 3-((E)Prop-1-enyl)-1,2,5-trimethoxyben-zene (4). To a stirred solution of 5 g (0.033 mol) of vanillin (**4a**) and 5.5 g (0.04 mol) of anhydrous K$_2$CO$_3$ in 50 mL of dry acetone was added allyl bromide (3.4 mL, 0.04 mol) dropwise.

The resulting mixture was refluxed for 2 h. After cooling the mixture was diluted by addition of water and the inorganic material was filtered off. The filtrate was extracted with ether. The organic extract was washed with 10% solution of NaOH and with water and next dried over MgSO$_4$. After the evaporation of the solvent, crude vanillin allyl ether was subjected to Claisen rearrangement. The ether was heated for 8 h at 185 °C in an oil bath. After cooling, the product was distilled under a reduced pressure to give 3 g of 3-methoxy-4-prop-2-enylben-zaldehyde (bp 100–107 °C, 5–7 mmHg). The resulting compound was oxidized with 50 mL of 3% solution (0.021 mol) of hydrogen peroxide. After 24 h, the mixture was neutralized with a 10% H$_2$SO$_4$ solution. Yellow precipitates were collected by filtration and recrystallized from hexane to give 2.9 g of 2-methoxy-6-prop-2-enylbenzene-1,4-diol.

A solution of 2.9 g (0.016 mol) of this product and 2.3 g (0.04 mol) of KOH in 40 mL of water was cooled to 10 °C and 1.5 mL (0.016 mol) of dimethyl sulfate was added dropwise. The mixture was warmed for 1 h at 80 °C. After cooling the organic layer was separated. The aqueous layer was extracted with ether. Organic extracts were combined and washed with 10% solution of NaOH and with water. After drying, the solvent was removed and the residue (2 g) was subjected to the next step.

A solution of 2 g (0.01 mol) of 1,3,5-trimethoxy-3-prop-2-enylbenzene in 25 mL of anhydrous ethanol and 32 g (0.6 mol) of KOH was heated 24 h under reflux. The mixture was diluted with water and extracted with ether. The ethereal solution was dried over MgSO$_4$, filtered and evaporated. The residual solid was treated with hexane, separated by filtration and purified by recrystallization (petroleum ether bp 40–60 °C) to give **4** (1.4 g, 70%): mp 44–45 °C; IR (CHCl$_3$) 3015, 1605, 1090 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 6.71 (dq, 1H, $J_1 = 1.68$ Hz, $J_2 = 15.9$ Hz); 6.55 (d, 1H, $J = 2.83$ Hz); 6.53 (d, 1H, ArH); 6.38 (d, 1H, $J = 2.84$ Hz); 6.25 (dq, 1H, $J = 6.6$ Hz, $J = 15.9$ Hz); 3.84 (s, 3H); 3.79 (s, 3H); 3.75 (s, 3H); 1.92 (dd, 3H, $J_1 = 1.67$ Hz, $J_2 = 6.59$ Hz); ^{13}C NMR 156.0 (C); 153.5 (C); 140.3 (C); 131.7 (C); 127 (C); 100.2 (CH); 125 (CH); 98.9 (CH); 60.9 (CH$_3$); 55.7 (CH$_3$); 55.4 (CH$_3$); 18.7 (CH$_3$); UV λ_{max} (nm) 220 ($\epsilon = 40262$), 254 (($\epsilon = 16073$), 304 ($\epsilon = 4145$); MS $m/z = 208$ (M$^+$, 100), 193 (91%), 178 (10%), 165 (40%), 150 (21%), 105 (18%).

Synthesis of 2-((E)Prop-1-enyl)-1,3,4-trimethoxyben-zene (5). To a solution of 1,2,4-trimethoxybenzene (3.024 g, 18 mmol) was added n-butyllithium (21.0 mL of 15% hexane solution) in 100 mL of dry hexane under neutral gas via syringe. After 1 h TMEDA (2.45 mL) and propanal (1.94 mL, 27 mmol) were added dropwise. The mixture was stirred for 10 min and 2 mL of water was carefully added. The resulting mixture was evaporated to dryness and the residue was extracted with methylene chloride (3 × 50 mL). The combined extracts were washed with 15% solution of H$_2$SO$_4$ and dried over MgSO$_4$. After evaporation to dryness the crude product was purified by column chromatography using carbon tetra-chloride–ethyl acetate mixture (30:1, v/v) as an eluent. 1.8 g of pure 1-(2,3,6-trimethoxyphenyl)-1-propanol was obtained.

The alcohol and anhydrous CuSO$_4$ (1.50 g, 9.3 mmol) in 50 mL of dry toluene were refluxed for 2 h. The inorganic salt was filtered off and the filtrate was evaporated to dryness. The residue was purified by column chromatography (carbon tetrachloride–ethyl acetate mixture, 20:1, v/v) to give compound **5** (1.4 g): IR (CHCl$_3$, cm^{-1}) 3020, 1610, 1050 cm^{-1}; UV λ_{max} (nm) 264 ($\epsilon = 15300$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 6.72 (d,1H, $J = 9.0$ Hz); 6.67 (dq, 1H, $J_1 = 5.57$ Hz, $J_2 = 16.0$ Hz); 6.58 (d, 1H, $J = 9.0$ Hz); 6.57 (d, 1H, $J = 9.0$ Hz); 3.83 (s, 3H); 3.80 (s, 3H); 3.78 (s, 3H); 1.94 (d, 3H, $J = 5.1$ Hz); ^{13}C NMR 152.0 (C); 147.6 (C); 147.2 (C); 131.3 (CH); 121.3 (CH); 121.1 (C); 110.2 (CH), 105.8 (CH), 60.1 (CH$_3$); 56.2 (CH$_3$); 55.8 (CH$_3$); 20.0 (CH$_3$); MS $m/z = 208$ (M$^+$, 100), 193 (32%), 179 (6%), 165 (13%), 150 (14%).

Hypolipidemic Activity. Wistar male rats weighing 200–250 g were purchased from the Pharmaceutical Research Institute, Warsaw, Poland. The animals were equally divided into groups of about 10 animals each and maintained at a

temperature of 24 ± 2 °C, 45% relative humidity, and 12 h periods changing of light and darkness. All rats were fed a high-cholesterol diet (Murigram enriched with cholesterol 1%, sodium cholate 0.2%, and olive oil 5%) for 7 days. Simultaneously, compounds diluted in oil were administered through a gastric intubation at 80 mg/kg once a day for the duration of the experiment. The group receiving clofibrate (150 mg/kg) served as a positive control. Compounds, diluted in oil, were adjusted so that the rats were administered a volume of 5 mL/kg of body weight. Rats fed with laboratory chow for the same duration as above were used as the non-cholesterol control group. The control group received a similar volume of vehicle oil. At the end of a 7-day period, each animal was fasted for 16 h and anesthetized with ether. Blood samples were collected through ocular puncture and centrifuged at 3000 rpm. Total cholesterol, HDL cholesterol, LDL cholesterol and triglicerides were determined using BioMerieux kits on a Shimadzu UV spectrophotometer.

Acute Pulmonary Thromboembolism Test in Mice. Male Swiss mice were obtained from the Pharmaceutical Institute, Warsaw, Poland. Mice were fed with conventional food and water ad libitum. The experiments were performed as described by DiMinno and Silver.[21] A pulmonary thromboembolic death or severe paralysis was induced in mice weighing 29.4 ± 2.6 g by a rapid administration into the tail vein of a mixture of collagen (25 μg/mouse) and epinephrine (27 μg/mouse). Mortality or paralysis was registered within 10 min after injection. Tested substances were given intraperitoneally as single doses 1 h before the thrombotic challenge in 0.1 mL of solvent/10 g of animal mass. Asarones were dissolved in miglyol 812, ketorolac in the mixture of DMSO (1 mL) and Söerensen phosphate buffer, pH = 7.0 (1.5 mL), acetylsalicylic acid in 0.3 M sodium acetate, collagen and epinephrine in 0.154 M sodium chloride. The vehicles were administered to animals in the same way as the substances studied, and their protection activities were treated as control values. The magnitude of the control data is similar to that reported previously.[15]

The results of the test were expressed as mortality (paralysis) rate, i.e., the total number of animals used and the percentage of those surviving.

In Vitro Platelet Aggregation. Platelet aggregation was measured by the Born method.[22] Human blood was collected by venipuncture from healthy male volunteers into 3.8% sodium citrate solution (volume ratio 9:1). The blood was centrifuged at $150g$ for 20 min to obtain platelet-rich plasma (PRP). A portion of PRP was further centrifuged at $2000g$ for 10 min to obtain platelet-poor plasma (PPP).

Aggregation was induced by ADP. The percentage aggregation was determined 6 min after the addition of the aggregating agent and was standardized by assuming that PPP represented 100% light transmission and PRP represented 0% light transmission. The agents studied were dissolved in 95% ethanol. The ethanol was reported not to influence aggregation in used volumes.[15] 1.0 μL of the solution of an agent of fixed concentration was added to 0.45 mL of PRP and placed in an aggregometer at 37 °C. Stirring rate was 1000 rpm. After 2-min preincubation ADP was added at a concentration inducing maximum aggregation and the changes in light transmission were recorded for 8 min. A maximum aggregatory effect was induced by adding 50 μL of 10 μM ADP in Tyrode solution to 0.45 mL of PRP.

Statistics. Student's t-test was used to determine the statistical significance of hypolipidemic activity of tested compounds. The χ^2 test was used for determining the significance of difference in the pharmacological response between the mice treated with the antithrombotic agents and the controls. In the platelet aggregation test in vitro arithmetical means and standard error of the mean (SEM) were determined.

Supporting Information Available: Purity data for α-asarone isomers. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Russel, R. The pathogenesis of atherosclerosis − an update. *N. Engl. J. Med.* **1986**, *314*, 488−500. (b) Gammill, R. B.; Day, Ch. E.; Schurr, P. E.; Khellin analogues. 1. General topological requirements for lipid-altering activity in furochromones. *J. Med. Chem.* **1983**, *26*, 1672−1674.

(2) Marx, J. L. The HDL: The good cholesterol carriers? *Science* **1979**, *205*, 677−679.

(3) (a) Mann, K. G.; Tracy, P. B.; Krishnaswamy, S.; Jenny, R. J.; Odegaard, B. H.; Nesheim, M. E. Platelets and coagulations. *Thrombosis and Haemostasis*; Leuven University Press: Brussels, 1987; pp 505−523. (b) Arniral, J., Farud, J. Thromboembolic diseases: biochemical mechanisms and new possibilities of biological diagnosis. *Semin. Thromb. Hemostas.* **1996**, *22* (Suppl. 1), 41−48. (c) Fuster, V.; Badimon, L.; Cohen, M.; Ambrose, J. A.; Badimon, J. J.; Chesebro, J. H. Insights into the pathogenesis of acute ischemic syndromes. *Circulation* **1988**, *77*, 1213−1220.

(4) Raines, E.; Ross, R. Smooth muscle cells and the pathogenesis of the lesions of atherosclerosis. *Br. Heart J.* **1993**, *69* (Suppl.), 30−37.

(5) Elwood, P.; Renaud, S.; Sharp, D.; Besewiick, A.; O'Brien, J.; Yarnel, W. Ischaemic heart disease and platelet aggregation. *Circulation* **1991**, *83*, 38−44.

(6) (a) Fitzgerald, G. A.; Meagher, E. A. Antiplatelet drugs. *Eur. J. Clin. Invest.* **1994**, *24* (Suppl. I), 46−49. (b) Fareed, J. Current trends in antithrombotic drug and device development. *Semin. Thromb. Hemost.* **1996**, *22* (Suppl. I), 3−8.

(7) Swies, J.; Robak, J.; Dıbrowski, L.; Dunies, Z.; Michalski, Z.; Gryglewski, R. J. Antiaggregatory effects of flavonoids in vivo and their influence on lipooxygenase and cyclooxygenase *in vitro*. *Pol. J. Pharm.* **1984**, *36*, 455−461.

(8) Chang, W.-Ch.; Hsu, F.-L. Inhibition of platelet aggregation and arachidonate metabolism in platelets by procyanidins. *Prostagland. Leuk. Essent. Fatty Acids* **1989**, *38*, 181−188.

(9) (a) Gracza, L.; Spaich, W. Analytische und biopharmazeutische Untersuchung transisoasaronhaltiger Preparate. *Planta Med.* **1978**, *33*, 160−169. (b) Belova, L. F.; Alibekov, S. D.; Baginskaya, A. I.; Sokolov, S. Ya.; Pokrovskaya, G. V. Asarone and its biological properties. *Farmakol.-Toksikol.* **1985**, *48*, 17−20. (c) Shirokova, E. A.; Segal, G. M.; Torgov, I. V. Synthesis (E) and (Z)-asarones and its analogues. *Bioorg. Chim.* **1985**, *2*, 270−272. (d) Hernandez, A.; Lopez, M. L.; Chamorro, G.; Mendoza-Figueroa, T. Inhibition of lipid synthesis and secretion in long-term culture of adult rat hepatocytes by α-asarone. *Planta Med.* **1993**, *59*, 121−124.

(10) (a) Enqiquez, R. G.; Chávez, M. A.; Jáuregui, F. Propenylbenzenes from Guatteria guameri. *Phytochemistry* **1980**, *19*, 2024−2025. (b) Diaz, F.; Muòoz, H.; Labarrios, F.; Chamorro, G.; Salazar, M.; Morelos, M. E.; Tamariz, J. Synthesis and hypolipidemic activity of some α-asarone analogues. *Med. Chem. Res.* **1993**, *3*, 101−109.

(11) Gomez, C.; Chamorro, G.; Chávez, M. A.; Martinez, G.; Salazar, M.; Pages, N. Effet de l' α-asarone sur l'hypercholesterolemie et la cholelithiasis experimentales. *Plant. Med. Phytother.* **1987**, *21*, 279−284.

(12) Menon, M. K.; Dandiya, P. C. The mechanism of the tranquilizing action of asarone from Acorus Calamus Linn. *J. Pharm. Pharmacol.* **1967**, *9* (3), 170−175.

(13) (a) Sagimoto, N.; Goto, Y.; Akao, N.; Kiucki, F.; Kondo, K. Mobility inhibition and nematocidal activity of asarone and related phenylpropanoids on second-stage larvae of Toxocara canis. *Biol. Pharm. Bull.* **1995**, *18*, 605−609. (b) Salazar, M.; Salaz, S.; Ulloa, V.; Mendoza, T.; Pages, N.; Chamorro, G. Teratogenic action of alpha-asarone in the mouse. *J. Toxicol. Clin. Exp.* **1992**, *12*, 149−154. (c) Chamorro, G.; Salazar, M.; Salazar, S.; Mendoza, T. Pharmacology and toxicology of Gatteria Guameri and alpha-asarone. *Rev. Invest. Clin.* **1993**, *45*, 592−604.

(14) (a) Gilman, H.; Thirtle, J. Dibenzofuran. XXI. Benzene and Biphenyl Intermediates for 1,9-Derivatives. *J. Am. Chem. Soc.* **1994**, *66*, 858−859. (b) Shulgin, A. T. The six trimethoxyphenylisopropylamines (Trimethoxyamphetamines). *J. Med. Chem.* **1966**, *9*, 445−449.

(15) Petrusewicz, J.; Turowski, M.; Foks, H.; Pilarski, B.; Kaliszan, R. Comparative studies of antiplatelet activity of nonsteroidal antiinflammatory drugs and new pyrazine CH- and NH-acids. *Life Sci.* **1995**, *56*, 667−677.

(16) Griffin, T. S.; Dock, E. L.; Brotherton, R. J.; Hall, I. H. The hypolipidemic activity of alkylamines and their boranes derivatives: structure−activity relationship in rodents. *Eur. J. Med. Chem.* **1991**, *26*, 517−527.

(17) Bisgaier, C.; Pape, M. High-density lipoprotein: are elevated levels desirable and achievable? *Curr. Pharm. Des.* **1998**, *4*, 53−70.

(18) (a) Hughes, T. A.; Elan, M. B.; Applegate, W. B.; Bond, M. G.; Hughes, S. M.; Wang, X.; Tolley, E. A; Bittle, J. C.; Stentz, F. B.; Kang, E. S. Postprandial lipoprotein responses in hypertriglyceridemic subjects with and without cardiovascular disease. *Metabolism* **1995**, *44*, 1082−1098. (b) Garduno, L.; Salazar, M.; Salazar, S.; Morelos, M. E.; Labarrios, F.; Tamariz, J.; Chamorro, G. A. Hypolipidemic activity of α-asarone in mice. *J. Ethnopharm.* **1997**, *55*, 161−163.

(19) Teitelbaum, P. Pharmacodynamics and Pharmacokinetics of Ticlopidine. In *Ticlopidine, Platelets and Vascular Disease;* Hass, W. K., Easton, J. D., Eds.; Springer-Verlag: New York, 1993; pp 27−40.

(20) Harushige, F.; Masatore, Y. Novel synthesis of 2,3,4-trimethoxy-1-propenylbenzene. *Yuki Gosei Kagaku Kyokai Shi* **1974**, *32*, 647−648.

(21) DiMinno, G.; Silver, M. J. Mouse antithrombotic assay: a simple method for the evaluation of antithrombotic agents in vivo. Potentiation of antithrombotic activity by alcohol. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 57−60.

(22) Born, G. V. R. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* **1962**, *194*, 927−929.